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NICOTINE INSECTICIDES
Part VI--SEARCH FOR SYNERGISTS (Continued)

By E. L. Mayer, R. H. Nelson, and Carl E. Robertson, Division of Stored Product Insect Investigations,[1/] Bureau of Entomology and Plant Quarantine, and J. J. Willaman, Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry

The search for compounds to replace some of the nicotine in insecticides and thereby make its use more economical has been continued. This paper presents the results of developmental laboratory work on adjuncts that appeared promising in preliminary screening tests, reported in Part V of this series (E-768). The compounds were furnished by the Eastern Regional Research Laboratory of the Bureau of Agricultural and Industrial Chemistry, and were tested against plant-feeding insects at the Anaheim, Calif., laboratory of the Bureau of Entomology and Plant Quarantine.

Materials and Methods

In most of the tests the nicotine was in the form of nicotine sulfate or nicotine bentonite dust. The nicotine sulfate was diluted with attapulgite. For the tests against the pomace fly the free alkaloid was dissolved in ethyl alcohol.

The dosages of nicotine alone ranged from 0.9 to 29.2 micrograms and of the adjunct alone from 1 to 29.6 micrograms per square centimeter. In the mixtures the dosages of nicotine ranged from 0.45 to 7.24 micrograms and of the adjunct from 1 to 18.1 micrograms per square centimeter.

1/ Formerly the Division of Control Investigations.

The insects and the foliage on which they were fed are as follows:

Insect	Stage	Foliage
Alder flea beetle (*Altica ambiens* (Lec.))	Fourth instar	Alder
Armyworm (*Cirphis unipuncta* (Haw.))	Third instar	Corn and barley
Bean aphid (*Aphis fabae* Scop.)	All stages	Nasturtium or rhubarb
California oakworm (*Phryganidia californica* (Pack.))	Fourth instar	Live oak
Celery leaf tier (*Phlyctaenia rubigalis* (Guen.))	Third instar	Swiss chard
Diamond back moth (*Plutella maculipennis* (Curt.))	do.	Collard
Dock beetle (*Gastrophysa cyanea* (Melsh.))	do.	Dock
English grain aphid (*Macrosiphum granarium* (Kby.))	All stages	Barley
Greenhouse thrips (*Heliothrips haemorrhoidalis* (Bouche))	Adults	Citrus
Pea aphid (*Macrosiphum pisi* (Kltb.))	First nymphal instar	Windsor bean
Pomace fly (*Drosophila melanogaster* (Meigen))	Adults	(Fed on sugar)
Variegated cutworm (*Peridroma margaritosa* (Haw.))	First instar	Broccoli

The materials were tested by infesting dusted foliage with first-instar larvae in cloth-covered vials and third- and fourth-instar larvae in 9-cm. petri dishes. The pea aphids were dusted directly on the plants on which they were feeding and were then confined in 16.5-cm. battery jars with cloth caps. The English grain aphids on barley were dusted and placed in petri dishes. The pomace flies were confined in shell vials lined with filter paper that had been dipped in ethyl alcohol containing the toxicants. The alcohol was allowed to evaporate completely before the flies were introduced. A few grains of granulated sugar were placed in the bottom of the vials as food for the flies. The greenhouse thrips were also confined in shell vials, the open ends of which were held securely to a dusted citrus leaf until mortality counts were made. Approximately 50 aphids and 30 of all other insects were used per test. Mortality counts were taken on the aphids after 2 days and on the other insects after 3 days.

Wadley's short-cut procedure (2) was used as the statistical approach in determining the presence of synergism, as was done in similar tests with phthalonitrile and pentachloroanisole (Mayer et al. 1). By this method the results are given in terms of the log ratio, which in this paper is the quotient of the difference between the log of the dosage at probit 5 (giving 50 percent mortality) for nicotine and the log of the same dosage for the nicotine equivalent, divided by the standard error. A log ratio of 2 is probably significant at the 5-percent level and 2.6 at the 1-percent level.

In Part V it was stated that where any mixture containing 5 percent of adjunct plus 2 percent of nicotine gave higher mortality than that given by the 5-percent nicotine standard alone against two or more insect species (from three to nine species were used), the adjunct was considered to be a possible synergist for nicotine. By this criterion the compounds given in table 1 of that paper were selected as having some promise, and developmental work was done on them. The results are presented in table 1 of this paper.

Only bis(p-chlorophenyl) sulfide and pentaerythritol diisobutyral showed definite synergism with most of the insects used; the other compounds showed not only no synergism but even antagonism. It is obvious that, if a more stringent criterion had been applied to the screening data, considerable developmental work could have been avoided.

In order to establish a basis for closer screening, two sets of data were compiled. One set, taken from tables 1 and 2 of Part V, included averages for the difference between the mortality caused by the mixture of nicotine and the adjunct (A) and that caused by the 5-percent nicotine alone (B), or for the difference between A and the sum of the mortalities caused by the 2-percent nicotine and the adjunct (C+D). The second set of data consisted of the average log ratios from table 1 of the present paper. Both sets of data, arranged in decreasing order of log ratios, are shown in table 2 of the present paper, together with the coefficients of correlation between the log ratios and the data from Part V. Since, to be significant, this coefficient should be three times the probable error, the coefficients from table 1 are significant but not those from table 2 (Part V). Therefore, the data in the latter table cannot be used in the selection of promising adjuncts. It should be pointed out that in obtaining these data the materials were tested against the pea aphid only, whereas in obtaining the developmental data as many as nine other species were used, and only three adjuncts were tested against the pea aphid; also that in Part V a 3.5-percent nicotine dust was used in table 2 and a 5-percent in table 1. So it is not strange that no significant correlation exists between the screening data on pea aphids and the developmental data on other species.

On the other hand, the data in table 1 of Part V are directly related to what may be expected when the Wadley technique is applied. The values for the screening data were plotted against those for the developmental work on square paper for the four comparisons above. Bis(p-chlorophenyl) sulfide and pentaerythritol diisobutyral consistently appeared in that quadrant having positive values for both abscissas and ordinates, an indication that these are the two best materials. If the average mortality against all insects for each material had been determined for table 1 of Part V and those compounds chosen for developmental work that averaged plus 10 or better for both A-B and A-(C+D), they would have shown but one good adjunct--bis(p-chlorophenyl) sulfide.

The question is: Why did so many of the compounds give antagonistic results (minus signs) in the developmental tests? A possible explanation is insect specificity. By the criterion previously explained, sesamin was chosen as a promising adjunct. In screening work this material was effective against the melonworm and the pea aphid, two species that were not used in developmental work. The armyworm and the diamondback moth were the only insects used in developmental work with sesamin. Antagonism was shown here as in the screening work. The use of different insects was unavoidable, since most of the screening tests were run on insects found in central Florida when the laboratory was located there, and all developmental work was done on California insects. The diamondback moth and the pea aphid were the only species tested in both places. In table 1 of Part V nearly all the plus signs (an indication of synergistic action) in column A-B are opposite Florida insects. Almost without exception, where the armyworm showed promise in the preliminary work it was also effective in the developmental study. In other words, if the same species of insects had been used in both the screening and developmental work, there would probably not have been so many materials showing antagonism. The insect used in the developmental work were susceptible only to pentaerythritol diisobutyral and bis(p-chlorophenyl) sulfide. There is therefore less specificity with these two materials, so that they are far superior to the others.

Even at the heaviest deposit of dust all allowed a moderate amount of feeding. Those containing the following adjuncts, however, allowed only a trace: Bis(p-chlorophenyl) sulfide, 2,4-dinitromesitylene, p-bromobenzenesulfonamide, 1,4-diphenylsemicarbazide, pentaerythritol diisobutyral, and phenyl sulfide.

Here again bis(p-chlorophenyl) sulfide and pentaerythritol diisobutyral were among the best materials.

In table 3 the adjuncts are placed into groups that show synergism, antagonism, or nonsignificance with nicotine when tested by the Wadley procedure. Neither the sulfate nor the bentonite of nicotine showed antagonism for bis(p-chlorophenyl) sulfide and pentaerythritol biisobutyral.

Literature Cited

(1) Mayer, E. L., McGovran, E. R., Talley, F. B., and Willaman, J. J.
1950. Tests for synergism between nicotine and phthalonitrile and between nicotine and 2, 3, 4, 5, 6-pentachloroanisole. Jour. Econ. Ent. 43: 533-537.

(2) Wadley, F. M.
1949. Short-cut procedure for error estimate in laboratory studies of synergism in insecticides. U.S. Bur. Ent. and Plant Quar. ET-275, 5 pp.

Table 1. --Results of developmental work on compounds that showed promise as synergists for nicotine in preliminary screening tests

Insect	Form of nicotine	Number of insects	Number of replicates 1/	Percent of toxicants		LD-50 (micrograms per square centimeter)		Log ratio 3/
				Nicotine	Adjunct	Nicotine	Adjunct 2/	
			Bis(p-chlorophenyl) sulfide					
Armyworm	Sulfate	300	3	2	2	3.8	9.0	4.6
	Bentonite	200	2	2	2	3.9	14	1.5
		200	2	2	2	5.5	6.6	2.9
Bean aphid:								
On nasturtium	Sulfate	622	2	1	1	1.4	-	.53
On rhubarb		1,005	4	1	1	1.9	-	2.8
	Bentonite	900	4	1	1	2.6	-	4.4
California oakworm	Sulfate	120	1	2	2	4.5	15.8	5.5
Diamondback moth	Sulfate	515	7	2	2	3	10.7	13.7
	Bentonite	400	4	2	2	3.9	18.1	11.2
Dock beetle	Sulfate	210	2	2	2	2.3	18.1	4.3
Pea aphid	Sulfate	860	3	2	2	3. 2	41.9	11.7

1/ Number of replicates presented were combined into a single calculation unless otherwise indicated

2/ A blank (-) indicates that the adjunct was not toxic.

3/ When log ratios were not calculable, pluses and minuses were assigned.

Table 1. --(Continued)

Insect	Form of nicotine	Number of insects	Number of replicates[1/]	Percent of toxicants		LD-50 (micrograms per square centimeter)		Log ratio[3/]
				Nicotine	Adjunct	Nicotine	Adjunct[2/]	
			2-Stilbazole					
Alder flea beetle	Sulfate	120	1	2	5	26.0	26.0	-10.0
Armyworm	Sulfate	270	3	2	2	3.7	13.5	-
		155	2[4/]	2	2	5.2	11.3	- 3.8
	Bentonite	285	2	2	2	2.6	23.5	- 7.3
		290	2[4/]	2	2	4.4	27.8	±
California oakworm	Sulfate	120	1	2	2	6.9	22	-
Diamondback moth	Sulfate	270	3	2	2	3	24	- 7.2
		90	1	2	2	7.1	-	-
		90	1	2	2	4	26.3	1.5
	Bentonite	200	2	2	2	4	38.5	-
		285	2	2	2	4.2	-	-
Dock beetle	Sulfate	200	2	2	2	3	56	4.1

4/ Average.

Table 1. --(Continued)

Insect	Form of nicotine	Number of insects	Number of replicates 1/	Percent of toxicants		LD-50 (micrograms per square centimeter)		Log ratio 3/
				Nicotine	Adjunct	Nicotine	Adjunct 2/	
			2, 4-Dinitromesitylene					
Alder flea beetle	Sulfate	280	3	2	5	21.5	15.0	±
Armyworm	Sulfate	180	2	2	2.5	3.9	28	-2.2
		180	2 4/	2	2	4.9	-	3.3
	Bentonite	205	2	2	2	4.2	42	+
		210	2 4/	2	2	5.2	-	+
Diamondback moth	Sulfate	270	4	2	2.5	2.2	12.8	+
		80	1	2	2	2.9	35	-2.1
	Bentonite	210	2	2	2	4.7	26.7	-1.7
		160	2 4/	2	2	4.1	30.1	-1.1
Dock beetle	Sulfate	345	3	2	2.5	2.5	16.8	3.5
Pea aphid	Sulfate	360	2 4/	2	2.5	1.6	12.5	-2.2
Pomace fly	Alkaloid	100	1	.1	.1	.08	.26 5/	-
			p-Bromobenzene sulfonamide					
Armyworm	Sulfate	440	4 4/	2	2	3.8	25	-2.2
Diamondback moth	Sulfate	335	4 4/	2	2	3.2	17.5	-1.3
	Bentonite	210	2	2	2	4.7	34.9	-3.5
		95	1	2	2	6.4	29	- -
		110	1	2	2	5.1	-	-1

5/ Alkaloid in solution.

Table 1.--(Continued)

Insect	Form of nicotine	Number of insects	Number of replicates[1/]	Percent of toxicants		LD-50 (micrograms per square centimeter)		Log ratio[3/]
				Nicotine	Adjunct	Nicotine	Adjunct[2/]	
1, 4-Diphenylsemicarbazide								
Armyworm	Sulfate	300	3	2	2	4.7	7.3	-1.3
	Bentonite	440	4[4/]	2	2	5.5	3.6	±
Diamondback moth	Sulfate	180	2	2	2	3.8	34	-4
	Bentonite	300	3[4/]	2	2	4.4	32.5	-2
Pentaerythritol diisobutyral								
Alder flea beetle	Sulfate	220	2[4/]	2	5	31	19	-
Armyworm	Sulfate	170	2	2	2.5	4.9	12.5	+
		105	1	2	2.5	2.72	9.6	+
		180	2	2	2.5	4	16.8	++
	Bentonite	520	4[4/]	2	2	4.6	20	1
California oakworm	Sulfate	100	1	2	2.5	8.6	25	-
Diamondback moth	Sulfate	200	2[4/]	1	2.5	8.5	9.8	-
		185	2[4/]	2	2.5	2.9	8.1	++
	Bentonite	280	2	2	2	3.1	11.7	3.1
		235	2[4/]	2	2	4	35	1.5
Dock beetle	Sulfate	390	3[4/]	2	2.5	2.5	22.5	3.6
Cetyl ether								
Armyworm	Sulfate	720	5	2	2	2.7	-	-5.6
	Bentonite	575	4	2	2	3.2	-	-5.5
Diamondback moth	Sulfate	430	3[4/]	2	2	3.8	2.7	.3

Table 1. --(Continued)

Insect	Form of nicotine	Number of insects	Number of replicates[1/]	Percent of toxicants		LD-50 (micrograms per square centimeter)		Log ratio[3/]
				Nicotine	Adjunct	Nicotine	Adjunct[2/]	
Sesamin								
Armyworm	Sulfate	575	4	2	2	2.1	-	-8
	Bentonite	575	4	2	2	3.1	-	-8
Diamondback moth	Sulfate	280	2	2	2	5.3	-	+
		145	1	2	2	2.9	33.2	-
Pentachlorocumene								
Armyworm	Sulfate	145	1	2	2	2	18.5	- -
		290	2[4/]	2	2	3.7	-	.5
	Bentonite	575	4[4/]	2	2	2.7	-	-4.5
2, 4-Dinitrophenyl 2, 3, 4, 5, 6-pentachlorophenyl ether								
Alder flea beetle	Sulfate	120	1	2	5	20	-	2.4
Armyworm	Sulfate	170	2	2	5	3.1	-	-4.3
		165	2	2	5	3.8	85	-2.7
	Bentonite	575	4	2	2	3.3	-	-10
California oakworm	Sulfate	120	1	2	2	9.5	45	-
Diamondback moth	Sulfate	90	1	2	2	5.7	-	+
		260	4	2	2	1.9	23.7	--
	Bentonite	310	4	2	2	2.1	16.2	-4.4
Dock beetle	Sulfate	100	1	2	2	2.7	-	5.4

Table 1.--(Continued)

Insect	Form of nicotine	Number of insects	Number of replicates[1/]	Percent of toxicants		LD-50 (micrograms per square centimeter)		Log ratio[3/]
				Nicotine	Adjunct	Nicotine	Adjunct[2/]	
			Phenyl sulfide					
Alder flea beetle	Sulfate	100	1	2	5	22.6	2.9	-2.5
Armyworm	Sulfate	210	2	2	2	2.8	3.1	-
	Bentonite	280	2	2	2	3.3	4.5	-
		100	1	2	2	5.7	8.2	-
California oakworm	Sulfate	100	1	2	2	9	12	-
Celery leaf tier	Sulfate	155	2	2	5	29.3	14.4	8.4
Diamondback moth	Sulfate	100	1	2	1.25	7.2	8.6	+
		300	3	2	2	3.8	2.5	-
	Bentonite	290	4	2	2	4.5	3.1	-
English grain aphid	Sulfate	900	2[4/]	1	1	1.8	10.1	±
Dock beetle	Sulfate	250	2	2	2	2.1	9.8	-
Greenhouse thrips	Sulfate	340	2[4/]	1	1	1.6	4	-
Pea aphid	Sulfate	220	1	1	1	1	37	-4
		295	2[4/]	2	2	1.3	16.1	-2
Variegated cutworm	Sulfate	230	1	2	2	14	4.5	-

Table 1. --(Continued)

Insect	Form of nicotine	Number of insects	Number of replicates1/	Percent of toxicants		LD-50 (micrograms per square centimeter)		Log ratio3/
				Nicotine	Adjunct	Nicotine	Adjunct2/	
			Tridecanenitrile					
Alder flea beetle	Sulfate	220	2 4/	2	5	11.5	26.0	-
Armyworm	Sulfate	200	2	2	2	3.7	19.3	-3.7
		200	2 4/	2	2	3.8	22.8	-2.7
	Bentonite	575	4 4/	2	2	4.2	28.5	-2.5
California oakworm	Sulfate	90	1	1	2.5	8.2	20	-
Diamondback moth	Sulfate	210	3	1	2.5	2.5	20	-
	Bentonite	530	4 4/	2	2	3.3	12.9	-
Dock beetle	Sulfate	220	2	1	2.5	2.7	9.4	6.1
		90	1	1	2.5	3.2	-	+
		90	1	1	2.5	1.4	8	-
			γ-Acetyl-γ-(2-cyanoethyl)pimelonitrile					
Armyworm	Sulfate	175	2	2	2	4.3	-	-4.8
		90	1	2	2	4	-	-
	Bentonite	290	2	2	2	2.4	-	-5.3
		290	2 4/	2	2	4.6	-	-
Diamondback moth	Sulfate	260	3	2	2	1.9	16.3	-2.2
		75	1	2	2	3.9	-	-
	Bentonite	560	4 4/	2	2	2.6	17.7	-
Dock beetle	Sulfate	180	2	2	2	1.4	-	3.3
		120	1	2	2	1.1	-	-

Table 1. --(Continued)

Insect	Form of nicotine	Number of insects	Number of replicates[1/]	Percent of toxicants		LD-50 (micrograms per square centimeter)		Log ratio[3/]
				Nicotine	Adjunct	Nicotine	Adjunct[2/]	
			2-Cyclohexylcyclohexylamine					
Alder flea beetle	Sulfate	220	2	2	5	60.0	-	4.7
Armyworm	Sulfate	270	3[4/]	2	2	4.3	16.0	-
	Bentonite	450	3	2	2	4.7	37	-3.6
		145	1	2	2	6	-	-2
Diamondback moth	Sulfate	300	4	2	2	3.1	12.2	-2.6
		120	1	2	2	4.3	18	2.6
	Bentonite	400	3	2	2	3.7	18.8	-2.3
		140	1	2	2	4.2	28.9	-1
Dock beetle	Sulfate	90	1	2	2	1.4	28.2	-
Pomace fly	Alkaloid	460	2[4/]	0.05	0.05	0.09	0.1[5/]	5.2

Table 2. --Comparison of log ratios obtained in present paper with screening data of Part V

Adjunct	Present paper		Part V				
			Average percent mortality				
			Table 1			Table 2 1/	
	Number of species	Average log ratio	Number of species	A-B	A-(C+D)	A-B	A-(C+D)
Bis(p-chlorophenyl) sulfide	6	6.8	4	25	16	-	-
Pentaerythritol diisobutyral	5	2.6	8	7	8	16	17
Phenyl sulfide	10	0	9	13	0	14	54
Tridecanenitrile	5	0	3	-14	-	4	39
2,4-Dinitromesitylene	6	0	8	-18	7	-13	14
2-Cyclohexylcyclohexylamine	5	0	3	-7	-	6	-
2,4-Dinitrophenyl 2,3,4,5,6-pentachlorophenyl ether	5	-2.3	4	-17	2	-	-
p-Bromobenzenesulfonamide	2	-2.6	7	-5	-30	-9	-8
1,4-Diphenylsemicarbazide	2	-3	7	4	-41	3	-72
Pentachlorocumene	1	-3.2	9	-22	-2	-23	-18
2-Stilbazole	5	-4.5	8	-11	-2	12	5
Cetyl ether	2	-5.5	7	-29	-20	14	15
Sesamin	1	-8	8	-21	-10	15	16
Coefficients of correlation of log ratios with screening data..............................				0.738 ±0.089	0.601 ±0.136	0.361 ±0.190	0.429 ±0.192

1/ Tests against the pea aphid only.

Table 3. --Number of tests in which adjuncts showed synergism, antagonism, or nonsignificance with nicotine sulfate or nicotine bentonite

Adjunct	Nicotine sulfate			Nicotine bentonite		
	Significant		Non-significant	Significant		Non-significant
	Synergism	Antagonism		Synergism	Antagonism	
Bis(p-chlorophenyl) sulfide	20	-	2	10	-	2
2-Stilbazole	2	9	3	-	2	6
2,4-Dinitromesitylene	5	5	7	-	-	9
p-Bromobenzenesulfonamide	-	4	4	-	3	1
1,4-Diphenylsemicarbazide	-	2	3	-	3	4
Pentaerythritol diisobutyral	7	-	8	2	-	6
Cetyl ether	-	5	3	-	4	-
Sesamin	-	4	3	-	4	-
Pentachlorocumene	-	1	2	-	4	-
2,4-Dinitrophenyl 2,3,4,5,6-pentachlorophenyl ether	2	8	2	-	8	-
Phenyl sulfide	2	4	14	-	-	7
Tridecanenitrile	2	4	8	-	4	4
γ-Acetyl-γ-(2-cyanoethyl)pimelonitrile	2	5	3	-	2	6
2-Cyclohexylcyclohexylamine	3	4	4	2 1/	7	1

1/ Tests with the nicotine alkaloid against the pomace fly only.